JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2003, p. 4404–4407 Vol. 41, No. 9
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.9.4404–4407.2003

Screening of Volunteer Students in Yaounde (Cameroon, Central Africa) for *Chlamydia trachomatis* Infection and Genotyping of Isolated *C. trachomatis* Strains

Antoinette Ngandjio,[1,2,3] Maithe Clerc,[2] Marie Christine Fonkoua,[1] Jocelyn Thonnon,[1] Friede Njock,[1] Regis Pouillot,[1] Françoise Lunel,[3] Christiane Bebear,[2] Bertille de Barbeyrac,[2] and Anne Bianchi[3,4]*

Centre Pasteur du Cameroun, Yaounde, Cameroon,[1] and Laboratoire de Bactériologie, Centre National de Référence des Infections à Chlamydia, Université de Bordeaux II, Bordeaux,[2] Institut Alfred Fournier, Paris,[3] and Laboratoire Départemental Conseil Général de Seine Saint Denis, Bondy,[4] France

Received 29 April 2003/Returned for modification 8 June 2003/Accepted 5 July 2003

The prevalence of *Chlamydia trachomatis* infection was 3.78% out of 1,277 volunteer students screened by direct fluorescence assay and Cobas Amplicor PCR. The infection was associated with the nonuse or inconsistent use of condoms in women ($P = 0.026$) and a previous sexually transmitted infection in men ($P = 0.023$). The most frequent genotypes determined by sequencing the *omp1* genes of 25 clinical isolates were E (44%) and F (20%), and some strains harbored mutations, but E genotype strains did not.

Chlamydia trachomatis, the leading cause of tubal infertility and ectopic pregnancy in women, is the most common sexually transmissible bacterial pathogen worldwide (4). Early detection, followed by correct treatment, is the key to reducing the risk of complications and the community-wide prevalence of sexually transmitted infections (STI) (4). The few studies of *C. trachomatis* that have been carried out in Cameroon targeted populations such as prostitutes (13), newborns (2), and pregnant women (16). Furthermore, nothing is known about the diversity of Cameroonian strains. Restriction fragment length polymorphism analysis (7, 17) and sequencing (5, 14) of the amplified *omp1* gene, which encodes the major outer membrane protein, were recently successfully used to genotype various *C. trachomatis* serovars.

This study was aimed at estimating the prevalence of *C. trachomatis* in volunteer students, identifying risk behaviors associated with the infection, and genotyping isolated strains. We also analyzed the relationship between genotypes and clinical signs reported by the students.

A total of 1,277 sexually active volunteer students (609 women and 668 men; mean age, 25.62 years) were screened for *C. trachomatis* infection between May and July 2001. The students provided information about their age, marital status, sexual behavior, past history of STI, and clinical signs. Follow-up care, treatment if necessary, and anonymity were guaranteed. The study was approved by the Cameroonian National Bioethics Committee.

Cervical and urethral specimens collected from women and men were used for *C. trachomatis* detection by direct fluorescence assay (DFA) and PCR. Dacron swabs for DFA were rolled over slides. The slides were air dried, fixed by incubation in methanol, and stained with the Syva MicroTrak *Chlamydia* direct specimen kit (Behring Diagnostics). The slides were examined under UV light at a magnification of ×500 for typical apple-green fluorescent elementary bodies (EB). The presence of more than five fluorescent EB was considered to be a positive result. For PCR detection, Bactopick swabs were immersed in 1.5 ml of 2-sucrose phosphate (2SP) transport medium. All 2SP media were maintained at 4°C during specimen collection and then aliquoted into three microtubes and frozen at −80°C within 4 h of collection. The Cobas Amplicor PCR test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* used was carried out according to the manufacturer's instructions (Roche Diagnostics, Meylan, France) 3 months later by technicians unaware of DFA results. The aim of this comparison was to evaluate the performance of the DFA test commonly use in Cameroon, which is cheaper than PCR.

If DFA and PCR gave discordant results, we carried out two further PCRs by the same method: one on the first lysate stored at −20°C and the other on a new extract performed under the conditions described by the manufacturer (Roche Diagnostics). Patients with at least two positive detections for the four tests performed (one DFA and three PCRs) were considered to be definitely infected by *C. trachomatis.*

The samples declared positive upon resolution of discrepancies were thawed and used to inoculate McCoy cell monolayers treated with cycloheximide. The chlamydial inclusion bodies were detected with a monoclonal antibody (Syva MicroTrak *Chlamydia* cell culture confirmation test; Behring Diagnostics). Strains (50 μl) were lysed by incubation in 100 μl of lysis buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 4.5 mM $MgCl_2$, 0.45% (wt/vol) Nonidet P-40, 0.45% (wt/vol) Tween 20, and 200 μg of proteinase K/ml at 56°C for 90 min and then at 95°C for 15 min. The *omp1* gene was amplified by PCR performed in a final volume of 50 μl with the primers CT1 (5′-GCC GCT TTG AGT TCT GCT TCC TC-3′) and CT5 (5′-ATT TAC GTG AGC AGC TCT CTC AT-3′) (Genset SA) (17). The program of amplification involved heating at 95°C for 5 min and 35 cycles of 1 min at 95°C, 1 min at

* Corresponding author. Mailing address: Laboratoire Départemental, Conseil Général de Seine Saint Denis, 41 Av. de Verdun, 93140 Bondy, France. Phone: 33 (0)1 48 48 16 34. Fax: 33 (0)1 48 50 74 62. E-mail: abianchi@ cg93.fr.

57°C, and 2 min at 72°C, followed by an extension period of 10 min at 72°C. PCR was performed in three separate rooms to avoid contamination. The PCR products were purified with the Wizard PCR Preps DNA purification system (Promega). The sequencing of the *omp1* gene was carried out on an ABI PRISM 377 genetic analyzer (PE Biosystems) using a BigDye sequencing kit (PE Biosystems) according to the manufacturer's instructions. All strains were sequenced by using the following three primers: CT1, VD2F (5′-CAT ATG CAA GAT GCT GAG ATG TTT AC-3′), and VD4F (5′-CTA TTG ATT ACC ATG AGT GGC AAG C-3′) (Genset SA) (11). When necessary, primers in reverse orientation, CT5 and VD3R (5′-AAA CTT GCT GAG ACC ATT TAA CTC C-3′) (Genset SA) (11), were used to confirm the nucleotide sequences. Consensus sequences for clinical isolates were determined by comparison with the American Type Culture Collection reference *omp1* nucleotide sequences obtained from GenBank. Clinical strains were sequenced once, and strains differing from the reference *omp1* sequence were reanalyzed by using a second PCR preparation to rule out the possibility of errors.

The collected data were analyzed statistically by χ^2 or two-tailed Fisher's exact tests, depending on the sample size. Values of P that were ≤ 0.05 were considered statistically significant. All analyses were performed with R software (10).

***C. trachomatis* prevalence and the relationship between infection and risk factors.** Ten specimens of the 1,277 tested (4 from women and 6 from men) had persistent PCR inhibitors (internal control remained negative) and were excluded from the study. All the inhibitory samples were DFA negative. DFA yielded positive results for 49 of 1,267 (29 of 605 from females and 20 of 662 from males) specimens, and PCR assay yielded positive results for 59 of 1,267 (26 of 605 from females and 33 of 662 from males) specimens. Concordant positive results were obtained for 38 specimens. DFA and PCR results were highly consistent (kappa coefficient = 0.69; 95% confidence interval [CI], 0.67 to 0.71). Eleven and 21 specimens yielded positive *C. trachomatis* detection only by DFA and PCR, respectively. Eight DFA-positive and 14 PCR-positive specimens, which were not confirmed positive by repeat testing, were considered false positive. On the basis of the discrepancy resolution, 48 (24 women and 24 men) of the 1,267 students were considered to have tested positive for *C. trachomatis*. The self-reported characteristics of the infected students are shown in Table 1. The overall prevalence of the infection was 3.78% (95% CI, 2.83 to 5.03%). The prevalence in women was 3.96% (95% CI, 2.61 to 5.93%), and that in men was 3.62% (95% CI, 2.38 to 5.42%). This prevalence of 3.78% is lower than the 38.3% reported for prostitutes (13) and the 6.9% reported for 508 students in Yaounde (3) but nonetheless demonstrates that *C. trachomatis* infection is widespread in Cameroon. The prevalence reported here is higher than the <2% prevalence reported for students in some developed countries (18). We believe that routine screening for STI in young people and the treatment of cases would decrease the frequency of STI, with major public health benefits, as up to 70% of infections in women are asymptomatic (20) and 10 to 25% of infected women suffer complications (9). Screening programs have consistently been followed by a marked reduction in chlamydial infection in Sweden (6) and in the United States (21). Chlamydial infection was a bit higher in students aged ≤25 years (4.21%) than in those over the age of 25 years (3.39%). The difference according to age was not statistically significant ($P > 0.05$), in contrast to what has been reported in many epidemiological studies (19). However, most of the students included were more than 20 years old. Forty-five (93.75%) of the 48 students with *C. trachomatis* infection reported having clinical signs, and only 3 (1 woman and 2 men) were asymptomatic. Of the students not infected with *C. trachomatis*, 85.89% (1,047 of 1,219) declared that they had symptoms. The major symptoms reported were vaginal discharge, vaginal itching, and lower abdominal pains in women, while in men, the symptoms were genital itching and urethral discharge.

Chlamydial infection was not significantly associated with the presence of at least one clinical sign ($P > 0.05$). The large number of uninfected students reporting symptoms and the relatively low prevalence of *C. trachomatis* infections suggest that unidentified STI are common and remain untreated in many students. This study design, based on voluntary recruitment, may also generate sampling bias, favoring the recruitment of students with clinical signs. Further studies with randomly recruited samples are required to determine the true proportion of young people with symptomatic or asymptomatic *C. trachomatis* infection. In univariate analysis (Table 2), the nonuse or inconsistent use of condoms was significantly asso-

TABLE 1. Characteristics of the infected students in Yaounde

Variable	No. of infected students/total (%)[a]	
	Women	Men
Age (yr)	24/602 (3.98)	24/652 (3.68)
≤25	18/373 (4.82)	10/292 (3.42)
>25	6/229 (2.62)	14/360 (3.88)
No. of sexual partners (in last yr)	24/605 (3.96)	24/662 (3.62)
0	0/26	3/60 (5.00)
1	13/375 (3.46)	6/220 (2.72)
2 to 5	11/200 (5.50)	12/344 (3.48)
More than 5	0/4	3/38 (7.89)
Change of sexual partner (in last 6 mo)	23/580 (3.96)	21/607 (3.45)
Usual partner	16/418 (3.82)	9/329 (2.73)
1 new partner	4/138 (2.89)	3/181 (1.65)
More than 1 new partner	3/24 (12.5)	9/97 (9.27)
Condom use (in the last yr)	24/581 (4.13)	21/605 (3.47)
Always	1/97 (1.03)	4/176 (2.27)
Never or occasionally	21/378 (5.55)	16/362 (4.41)
Never but the same partner	2/106 (1.88)	1/67 (1.49)
History of STI (in the last yr)	24/605 (3.96)	24/662 (3.62)
No	18/472 (3.81)	11/452 (2.43)
Yes	6/133 (4.51)	13/210 (6.19)
Marital status	24/605 (3.96)	24/662 (3.96)
Single	20/522 (3.83)	21/594 (3.53)
Married or cohabitating	4/83 (4.81)	3/68 (4.41)
Symptom category	24/605 (3.80)	24/662 (3.96)
Asymptomatic	1/67 (1.49)	2/108 (1.85)
Urogenital symptoms	23/538 (4.27)	22/554 (3.97)

[a] Differences in the total numbers of students for certain variables are due to missing data.

TABLE 2. Association of STI risk factors and genital chlamydial infections in students

Variable	Women				Men			
	% with infection for which variable was:		OR (95% CI)	P^b	% with infection for which variable was:		OR (95% CI)	P
	Present	Absent			Present	Absent		
Age ≤25 yr	4.82	2.62	1.88 (0.74–4.82)	0.204	3.42	3.88	0.88 (0.38–2.00)	0.836
More than one sexual partner in last yr	5.39	3.24	1.70 (0.75–3.87)	0.269	3.92	3.21	1.23 (0.53–2.85)	0.679
A new sexual partner in the last 6 mo	4.32	3.82	1.13 (0.46–2.81)	0.813	4.31	2.73	1.60 (0.67–3.86)	0.373
Lack of or inconsistent condom use[a]	5.55	1.50	3.89 (1.15–13.21)	0.026*	4.41	2.05	2.20 (0.80–6.09)	0.172
STI in last 12 mo	4.51	3.81	1.19 (0.46–3.06)	0.801	6.19	2.43	2.62 (1.16–6.01)	0.023*
Single	3.83	4.81	0.79 (0.26–2.36)	0.557	3.53	4.41	0.79 (0.23–2.73)	0.728
Clinical signs	4.27	1.49	2.95 (0.39–22.19)	0.502	3.97	1.85	2.19 (0.51–9.46)	0.402

[a] Only students without steady sexual partners were considered.
[b] *, significant correlation.

ciated with infection (odds ratio[OR], 3.89; 95% CI, 1.15 to 13.21; $P = 0.026$) in women. Encouraging condom use may also help to control other STI and human immunodeficiency virus (HIV) infection. Several studies have demonstrated that HIV infection is associated with *C. trachomatis* (3, 8). In men, a history of STI in the last 12 months was significantly associated with the risk of genital *C. trachomatis* infection (OR, 2.62; 95% CI, 1.16 to 6.01; $P = 0.023$).

Analysis of the sequence of the *C. trachomatis omp1* gene. The *omp1* genes of 25 strains were successfully amplified and sequenced. Our sequence data were clear and showed no sign of mixed infections. Despite the small number of strains sequenced, typing these strains revealed them to have diverse genotypes. The heterogeneity of *C. trachomatis* in this population is consistent with that reported in other areas (22, 23). The most common *C. trachomatis* genotype identified was E ($n = 11$; 44%), followed by F ($n = 5$; 20%) and D/Da ($n = 5$; 20%). Three genotype J (12%) strains and one genotype G (4%) strain were identified. The predominance of genotype E in this study and others (15) suggests that this genotype may have a real biological advantage over other genotypes. The *omp1* gene nucleotide sequences of all the genotype E strains and their corresponding amino acid sequences were identical to those for the entire *omp1* gene of the reference E/Bour/Peterson strain. Some sequence variations were found within the various non-E genotypes and did not involve the same nucleotides, likely due to the limited number of strains sequenced (Table 3). All the genotype J strains found in this study presented nucleotide substitutions. One had a silent substitution at the same position (residue 369; C→T) as that described by Jurstrand et al. (12).

Few studies have addressed the possible correlation between specific genotypes and clinical signs. In our study, no association between genotypes and specific clinical symptoms, for either men or women, was found ($P > 0.05$). However, Van Duynhoven et al. found that genotypes H and J were most commonly associated with urethral discharge and dysuria in men, whereas the F and G group genotypes were most frequently associated with lower abdominal pains in women (23). It was also recently suggested that serovar G was associated with the subsequent development of cervical squamous cell carcinoma (1).

In conclusion, this study constitutes a step toward the control of *C. trachomatis* infection in Cameroon. DFA in our hands was a good technique. Epidemiological data are required to assess the impact of the infection and to set up

TABLE 3. Mutations found in the strains isolated from students, as detected by comparison with prototypes in GenBank

Wild strain no., genotype	No. of mutations	Mutation	Position	Amino acid change	Domain[a]	Type strain, GenBank accession no.
8, D	2	C→T	423	Silent	CD II	D/UW-3/Cx, AE001338
		G→A	510	Silent	CD II	
20, Da	1	A→G	235	N→D	CD I	D/11/cal-8, X62920
26, G	1	T→G	1003	S→A	VD IV	G/UW57/Cx, AF063199
2, F	1	G→A	780	Silent	VD III	F IC-Cal-3, X52080
22, F	1	T→G	587	V→G	CD III	F IC-Cal-3, X52080
21, J	1	A→G	107	D→G	CD I	J/UW36/Cx, AF063202
27, J	2	C→T	369	Silent	VD I	J/UW36/Cx, AF063202
		T→C	554	V→A	VD II	
28, J	3	G→A	607	A→T	CD III	J/UW36/Cx, AF063202
		G→A	706	E→K	CD III	
		T→C	888	Silent	CD IV	

[a] CD, constant domain; VD, variable domain.

screening programs. The characterization of *C. trachomatis* strains may provide valuable information about circulating strains and variants.

This work was supported by a grant from Agence Universitaire de la Francophonie, Mission Française de Coopération au Cameroun, and Centre Pasteur du Cameroun.

We thank P. Martin and P. Cunin for their contribution to the initiation of this study, P. Perez for epidemiological advice, and A. Charron and P. Gonzalez for technical assistance. We also thank the Student Medical Center (University of Yaounde I), which agreed to receive students, and Roche Diagnostic, which supplied some of the PCR reagents.

REFERENCES

1. **Anttila, T., P. Saikku, P. Koskela, A. Bloigu, J. Dillner, I. Ikaheimo, E. Jellum, M. Lehtinen, P. Lenner, T. Hakulinen, A. Narvanen, E. Pukkala, S. Thoresen, L. Youngman, and J. Paavonen.** 2001. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA **285:**47–51.
2. **Buisman, N. J., T. A. Mwemba, G. Garrigue, J. Durand, J. Stilma, and T. van Balen.** 1988. *Chlamydia* ophthalmia neonatorum in Cameroon. Doc Ophthalmol. **70:**257–264.
3. **Buve, A., H. Weiss, M. Laga, E. Van Dyck, R. Musonda, L. Zekeng, M. Kahindo, S. Anagonou, L. Morison, N. Robinson, and R. Hayes.** 2001. The epidemiology of gonorrhoea, chlamydial infection and syphilis in four African cities. AIDS **15**(Suppl. 4)**:**S79–S88.
4. **Centers for Disease Control and Prevention.** 1993. Recommendations for the prevention and management of *Chlamydia trachomatis* infections, 1993. Morb. Mortal. Wkly. Rep. Recomm. Rep. **42:**1–39.
5. **Dean, D., J. Schachter, C. Dawson, and R. Stephens.** 1992. Comparison of the major outer membrane protein variant sequence regions of B/Ba isolates: a molecular epidemiologic approach to *Chlamydia trachomatis* infections. J. Infect. Dis. **166:**383–392.
6. **Egger, M., N. Low, G. D. Smith, B. Lindblom, and B. Herrmann.** 1998. Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: ecological analysis. Br. Med. J. **316:**1776–7129.
7. **Frost, E., S. Deslandes, S. Veilleux, and D. Bourgaux-Ramoisy.** 1991. Typing *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. J. Infect. Dis. **163:**1103–1107.
8. **Ghys, P. D., K. Fransen, M. Diallo, V. Ettiegne-Traore, I. Coulibaly, K. Yeboue, M. Kalish, C. Maurice, J. Whitaker, A. Greenberg, and M. Laga.** 1997. The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Cote d'Ivoire. AIDS **11:**F85–F93.
9. **Grun, L., J. Tassano-Smith, C. Carder, A. M. Johnson, A. Robinson, E. Murray, J. Stephenson, A. Haines, A. Copas, and G. Ridgway.** 1997. Comparison of two methods of screening for genital chlamydial infection in women attending in general practice: cross sectional survey. Br. Med. J. **315:**226–309.
10. **Ihaka, R., and R. Gentleman.** 1996. R: a language for data analysis and graphics. J. Comput. Graph. Stat. **5:**299–314.
11. **Ikehata, M., K. Numazaki, and S. Chiba.** 2000. Analysis of *Chlamydia trachomatis* serovars in endocervical specimens derived from pregnant Japanese women. FEMS Immunol. Med. Microbiol. **27:**35–41.
12. **Jurstrand, M., L. Falk, H. Fredlund, M. Lindberg, P. Olcen, S. Andersson, K. Persson, J. Albert, and A. Backman.** 2001. Characterization of *Chlamydia trachomatis omp1* genotypes among sexually transmitted disease patients in Sweden. J. Clin. Microbiol. **39:**3915–6008.
13. **Kaptue, L., L. Zekeng, S. Djoumessi, M. Monny-Lobe, D. Nichols, and R. Debuysscher.** 1991. HIV and *Chlamydia* infections among prostitutes in Yaounde, Cameroon. Sex. Transm. Infect. **67:**143-145.
14. **Lampe, M. F., R. Suchland, and W. E. Stamm.** 1993. Nucleotide sequence of the variable domains within the major outer membrane protein gene from serovariants of *Chlamydia trachomatis.* Infect. Immun. **61:**213–219.
15. **Morré, S. A., L. Rozendaal, I. G. M. van Valkengoed, A. J. P. Boeke, P. C. van Voorst Vader, J. Schirm, S. de Blok, J. A. R. van den Hoek, G. J. J. van Doornum, C. J. L. M. Meijer, and A. J. C. van den Brule.** 2000. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection: an association with clinical manifestations? J. Clin. Microbiol. **38:**2292–2296.
16. **Ngassa, P. C., and J. Egbe.** 1994. Maternal genital *Chlamydia trachomatis* infection and the risk of preterm labor. Int. J. Gynaecol. Obstet. **47:**241–246.
17. **Rodriguez, P., A. Verkris, B. de Barbeyrac, B. Dutilh, J. Bonnet, and C. Bebear.** 1991. Typing of *Chlamydia trachomatis* by restriction endonuclease analysis of the amplified major outer membrane protein gene. J. Clin. Microbiol. **29:**1132–1136.
18. **Rogstad, K. E., S. M. Bates, S. Partridge, G. Kudesia, R. Poll, M. A. Osborne, and S. Dixon.** 2001. The prevalence of *Chlamydia trachomatis* infection in male undergraduates: a postal survey. Sex. Transm. Infect. **77:**111–113.
19. **Santer, M., P. Warner, S. Wyke, and S. Sutherland.** 2000. Opportunistic screening for *Chlamydia* infection in general practice: can we reach young women? J. Med. Screen. **7:**175–176.
20. **Schachter, J., E. Stoner, and J. Moncada.** 1983. Screening for chlamydial infections in women attending family planning clinics. West. J. Med. **138:** 375–379.
21. **Stamm, W. E.** 1998. Expanding efforts to prevent chlamydial infection. N. Engl. J. Med. **339:**768–770.
22. **Sturm-Ramirez, K., H. Brumblay, K. Diop, A. Guèye-Ndiaye, J.-L. Sankalé, I. Thior, I. N′Doye, C.-C. Hsieh, S. Mboup, and P. J. Kanki.** 2000. Molecular epidemiology of genital *Chlamydia trachomatis* infection in high-risk women in Senegal, West Africa. J. Clin. Microbiol. **38:**138–145.
23. **van Duynhoven, Y., J. Ossewaarde, R. Derksen-Nawrocki, W. van der Meijden, and M. van de Laar.** 1998. *Chlamydia trachomatis* genotypes: correlation with clinical manifestations of infection and patients' characteristics. Clin. Infect. Dis. **26:**314–322.